have greatly reduced inflammatory pain sensation. More recently, mutations in *SCN11A* encoding human $Na_V1.9$ have been associated with either loss of pain perception, or familial episodic pain and painful peripheral neuropathy. Here we elucidate the functional consequences of a novel heterozygous $Na_V1.9$ mutation (L1302F) discovered in a female diagnosed with congenital insensitivity to pain (CIP). The mutation was stably expressed in ND7/23 cells and whole-cell currents were elicited with 50 ms pulses from −120 to +40 mV from a holding potential of −140 mV in the continuous presence of 150 nM TTX to block endogenous TTX-sensitive sodium currents. Cells expressing $Na_V1.9$-WT exhibited whole-cell current that peaked at −40 mV (31.2 ± 3.5 pA/pF, n=10) with a voltage-dependence of activation defined by V½ = −61.8 ± 1.5 mV and slope factor (k) of 6.1 ± 0.4 (n=10). By contrast, $Na_V1.9$-L1302F whole-cell current peaked at −70 mV (39.5 ± 8.7 pA/pF, n=12) and exhibited a significantly hyperpolarized voltage-dependence of activation (V½ = −86.6 ± 1.1 mV; k = 6.5 ± 0.4; n=12). These results initially appeared to indicate that the mutation potentiates channel function by enabling activation at more negative membrane potentials. However, when currents were recorded using a holding potential of −90 mV, mutant channel activity was reduced substantially by ~95% (1.5 ± 0.3 pA/pF @ −70 mV, n=7), whereas the WT channel had more preserved activity ~50% (15.6 ± 3.1 pA/pF @ −40 mV, n=6). These results suggest that the effect of this mutation is more likely a loss-of-function under physiological conditions, and this will reduce neuronal excitability leading to impaired pain sensation.

2913-Pos Board B343

Infant Sudden Death: Novel Mutations Responsible for Impaired Nav1.5 Channel Function

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Sudden infant death syndrome (SIDS) is the leading cause of mortality in apparently normal infants. During 2008 to 2012, the New York City Office of Chief Medical Examiner (OCME) examined 274 cases of sudden unexplained death (SUD) of which 141 were infants below 1 year of age, with ~93% of these less than 6 months of age at the time of death. Several ion channelopathies were found during genetic screening. An African-American/Hispanic girl who died suddenly in her sleep at the age of 5 weeks carried two SCN5A mutations: c.5494 C>G and c.5830 C>T, which respectively introduces a missense mutation Q1832E and an early stop codon R1944X in the distal C-terminus of the cardiac Na^+ channel -subunit, Nav1.5. HEK-293 cells were transfected with cDNAs of wild-type Nav1.5, Nav1.5-Q1832E, Nav1.5Δ1944 (the C-terminal truncation) or a cDNA with both mutations (Nav1.5-Q1832E-Δ1944) and were subjected to whole-cell patch clamping. The peak Nav1.5-Q1832E current was reduced by almost 10-fold (e.g. at −20mV the wild-type Nav1.5 was 283 ± 49.1 pA/pF, n=8 cf. −31 ± 11.8 pA/pF, n=4, for Nav1.5-Q1832E, $p<0.001$), whereas Nav1.5Δ1944 and Nav1.5-Q1832E-Δ1944 currents were not significantly different from wild-type. The inactivation time constants were unaffected by any of the mutations (e.g. at −10mV, 1 and 2 respectively were 1.3 ± 0.15 and 7.7 ± 0.87 ms, n=8 for wild-type cf. 1.1 ± 0.21 and 7.5 ± 1.61 ms, n=4, for Nav1.5-Q1832E). No significant differences were observed for the time course of the recovery from inactivation or the voltage dependence of the activation and inactivation kinetic variables. Preliminary biotinylation experiments suggest that the Nav1.5-Q1832E surface expression was unaltered compared to wild-type, suggesting a defect independent of trafficking. In conclusion, the Q1832E mutation was sufficient to produce a severely dysfunctional Nav1.5 channel, which may have been contributing to the victim's sudden death.

2914-Pos Board B344

Biophysical and Molecular Analysis of the Sodium Current in Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes

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Background: Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have been used for safety pharmacology and to investigate genetic diseases affecting cardiac ion channels. We examined I_{Na} in hiPSC-CMs and determined its contribution to action potentials (APs) recorded from monolayers of hiPSC-CMs.
Methods: Commercially available hiPSC-CMs were plated at high density to form monolayers or low density to yield single cells. AP recordings from monolayers were made using high resistance electrodes at 36°C. Whole cell patch clamp was used to record I_{Na} in single hiPSC-CMs at room temperature.
Results: AP recordings showed spontaneous activity with a maximum diastolic potential (MDP)= −69.2 ± 1.4 mV and upstroke velocity=41.9 ± 6.7 V/s. Application of tetrodotoxin resulted in a slowing of the AP rate but had little effect on AP upstroke or duration. In single hiPSC-CMs, a large I_{Na} was recorded when external Na^+ was reduced to 40 mM (73.6 ± 6.18 pA/pF). Recovery of I_{Na} (hp= 120 mV) was very fast; at hp= 80 mV, recovery of I_{Na} was slower and the size of peak I_{Na} was greatly reduced (27.0 ± 3.38 pA/pF). Molecular analysis showed that SCN5A was the predominate Na^+ channel subtype in both adult and iPSC-CMs. In adddition, we found that iPSC-CMs express both the fetal (exon 6A) and adult (exon 6) isoforms of SCN5A. Action potential clamp experiments showed that application of a ventricular or Purkinje cell waveform to the same hiPSC-CM elicited a large I_{Na} while application of a SA node waveform elicited no I_{Na}.
Conclusion: A large I_{Na} is present in hiPSC-CM but its contribution to the AP upstroke is minimal. The depolarized MDP coupled with the presence of phase 4 depolarization results in a take-off potential of −60.6 ± 1.7 mV which inactivates the majority of Na^+ channels.

2915-Pos Board B345

Nav1.5 C-Terminal Domains Influence Calcium Regulation of Fast Inactivation Separately from Calmodulin Interaction

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The cardiac sodium channel (Nav1.5) has a complex 'intracellular Ca^{2+} sensing apparatus' within its C-terminal domain (CTD) consisting of a partial EF-hand domain (CTD-EF) and a calmodulin (CaM) binding IQ motif (CTD-IQ). There are additional CaM binding motifs within the DIII-DIV linker. Variation in intracellular Ca^{2+} concentration influences the voltage-dependence of steady-state fast inactivation (SSI) by an unclear mechanism. Here, we mutated 16 key residues implicated in the interactions between CTD-EF and CTD-IQ, CTD-IQ and CaM, or DIII-DIV and CaM and explored their impact on NaV1.5 function and biochemistry. We used quantitative yeast-two-hybrid assays to measure effects of mutations on the interaction between the full length CTD on CaM, and evaluated SSI in high (1 μM free Ca^{2+}) and low $[Ca^{2+}]$i conditions. Using either BAPTA or HEDTA as chelator, 1 μM free $[Ca^{2+}]$i was sufficient to shift SSI (elicited by 50 ms prepulses) towards depolarized potentials. We observed that 3 of 5 CTD-IQ mutations (F1912A, A1924T and IQ/AA) strongly reduced the interaction between the CTD and CaM. However, these mutations did not affect the $[Ca^{2+}]$i effect on SSI suggesting that SSI $[Ca^{2+}]$i sensitivity does not depend on CaM binding to the CTD-IQ. Unexpectedly, single and combination mutations of the CTD-EF (L1786A, F1791A, Q1807A, L1862A, E1788A-D1790A-D1792A-E1799A, E1804A-D1802A) diminished the CTD-CaM interaction, and some of these mutations (L1786A, E1788A-D1790A-D1792A-E1799A, E1804A-D1802A) also suppressed the $[Ca^{2+}]$i effect on SSI. Indeed, the only mutations we studied that blunted the $[Ca^{2+}]$i effect on SSI were within the CTD-EF. These results suggest that the CTD-EF influences NaV1.5 $[Ca^{2+}]$i sensitivity and mutations in this domain can also alter the interaction of the CTD with CaM, but CaM interaction with the CTD-IQ is not required for the effect of intracellular Ca^{2+} on inactivation.

2916-Pos Board B346

CaMKII-Dependent Regulation of Cardiac Sodium Channel

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Voltage-gated Na^+ channels are key determinants of conduction, action potential profile and refractoriness in mammalian myocardium. Na^+ channels are regulated by a number of protein kinases and alterations in phosphorylation are associated with the phenotypic expression of inherited and acquired heart diseases. Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) phosphorylates $Na_V1.5$ at multiple sites in the domain I-II linker, with effects on channel gating. The CaMKII phosphorylated sites we identified by mass spectrometry (MS) include serines 459, 460, 484, 539, 571, 664, 667 and threonine 486 in the I-II linker and serines 1925, 1937 and 1969 in the carboxyl-Terminus (CT). In addition we evaluated the effect of CAMKII phosphorylation on $Na_V1.5$-S528A and $Na_V1.5$-R526H channels in which direct phosphorylation by PKA is abolished (Aiba, 2014). In Wild-type $Na_V1.5$ channels, acute exposure to CaMKII increased the peak current,

produced a-10 mV shift in the availability curve, slowed recovery from inactivation and increased the late Na current ($I_{Na\text{-}L}$). These changes were prevented by inclusion of the CaMKII inhibitor peptide, AIP in the pipette or partially prevented by exposure to the PKA inhibitor PKI. $Na_V1.5$-S571A, $Na_V1.5$-S526H and $Na_V1.5$-S528A channels eliminated the CaMKII induced shift in inactivation, slowing of recovery and increase in $I_{Na\text{-}L}$ S571D or S528D mimicked the effects of phosphorylation. In $Na_V1.5$-S1969A channels exposure to CAMKII increases $I_{Na\text{-}L}$ compared to $Na_V1.5$-WT with a further increase in $I_{Na\text{-}L}$ in the presence of CaMKII; however, the steady state inactivation curve is not shifted. These data suggest the presence of functional phosphorylation sites in the CT and the interaction of CaMKII and PKA in the I-II linker. The cross talk between CaMKII and PKA modulation of the channel may have important implications for electrophysiological properties of the heart.

2917-Pos Board B347

Recruitment of Calmodulin to the Tail of the Voltage-Gated Sodium Channel Nav1.2

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Voltage-gated sodium channels (NaV) found in excitable cells are responsible for the rising phase of action potentials. These multi-domain transmembrane proteins are regulated by calmodulin (CaM), a highly conserved eukaryotic protein that mediates many calcium-triggered signaling events. Inactivation of sodium channels depends on CaM-mediated feedback during repolarization. In the neuronal sodium channel NaV1.2, CaM binds at least two well-separated sites: an intracellular ''inactivation'' loop between domains DIII and DIV, and an IQ motif [IQRAYRRYLLK] in the cytosolic C-terminal tail. The IQ motif is hypothesized to recruit calcium-free (apo) CaM, making it available to move to the III-IV linker after an influx of calcium. Despite a high degree of sequence identity, the equilibrium constants for CaM binding to nine human NaV IQ motifs span more than 3 orders of magnitude. Apo CaM binds to the NaV1.2 IQ motif with a dissociation constant (Kd) of ~6 nM, while the Kd for binding the NaV1.9 IQ motif is ~ 4 μM. Mutational analysis within the IQ motif has not been sufficient to explain the full range of CaM-binding affinities observed for human NaV sequences. Thus, we hypothesized that isoform-specific differences in upstream sequences were making energetically significant contributions to the free energy of binding CaM to NaV1.2. The roles of these residues are being investigated by monitoring CaM binding to biosensors containing mutant sequences of sodium channels bracketed by auto-fluorescent proteins YFP and CFP. Residue-specific information obtained by NMR will provide structural insight into the contributions of residues in the binding interface formed by NaV IQ motif sequences binding to calmodulin from multiple eukaryotes. NIH R01 GM57001.

2918-Pos Board B348

Coupling Compartmental Models to Live Neurons to Investigate Action Potential Mechanisms

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In mammalian central neurons, action potentials are initiated in the axonal initial segment (AIS) by Nav1.2 and Nav1.6 channels, and shaped and terminated by other voltage-gated ion channels. From the AIS, the AP travels down the axon towards the presynaptic site, but also back-propagates towards the soma. The role of axonal sodium channels in AP initiation and propagation is still incompletely understood, mostly because it is difficult to record from these channels at the AIS. Instead, most experimental evidence of axonal activity is obtained indirectly, from electrical recordings at the soma. To better understand these mechanisms, we developed a real-time computational procedure where a compartmental model of the axon is coupled to a live neuron using dynamic clamp. The properties of this computational model (e.g., spatial distribution and kinetics of ion channels) are varied until the firing activity of the hybrid construct (neuron + axon compartmental model) best matches the normal activity of the neuron.

2919-Pos Board B349

Optimizing a Nav1.5 Markov-Model with a Genetic Algorithm

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Background: Markov models of cardiac voltage-gated Na^+ (Na_V) channels have been widely used for cell and tissue simulations of cardiac electrophysiology. These $Na_V1.5$ models are of varying complexity. Simple models with few states cannot account for all of the kinetics observed with multiple protocols. Increasingly complex models become computationally infeasible for multi-scale simulations and their results are difficult to interpret.
Methods: Following previously published work for the neuronal Na^+ channel, we have implemented a genetic algorithm to optimize model topology and rate parameters for the cardiac sodium channel. The advantage of this approach is a flexible topology, with unnecessary states and edges removed in favor of model plausibility and computational speed. We improved this model by parallelizing the computation and including subpopulations with random mutation rates. Patch-clamp data for model parameterization was collected by recording transiently transfected HEK293T cells expressing $Na_v1.5$ channels 24 hours post-transfection. The temperature of the recordings was controlled at various temperatures ranging from room temperature to physiological temperatures. Models were successfully fit to patch-clamp protocols, consisting of activation curves, inactivation, recovery from inactivation, and activation current traces.
Results: The final optimization employed 32 subpopulations each with 50 members and was run for 2000 generations. After 2 x 10^6 models were evaluated, the algorithm yielded a model composed of 8 states and 10 interconnecting edges. The model was able to reproduce 20ms, 100ms, and 1000ms inactivation holds, activation, and recovery from inactivation protocols with high fidelity. Fast kinetic data was reproduced by fitting traces for −30mV, −10mV, and 10 mV directly. This novel model fits a wide range of experimentally collected data and contains significantly fewer parameters than current, widely used models.

2920-Pos Board B350

Rate Constant Models cannot Describe Movement of Charged Atoms or Molecules

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Rate constant models built on laws of mass action are used widely to describe movement of ions and electric current through channels, and chemical reactions of charged substrates. But mass action laws are derived from conservation of mass and say nothing about electric charge. Laws of electricity (Maxwell's equations: a generalization of Kirchoff's current law) are about conservation of charge, not mass. In classical rate models, flows of ions are not correlated by laws of electricity. But Maxwell's equations strongly correlate flows of charge (including displacement current), **without known exception**, within one part in 10^{18} or so. Correlation coefficients (describing correlated flows of charges in rate models) should then be nearly one, something like 0.999 999 999 999 999 999. Correlations of charge movement are ignored in classical rate models, so **classical rate models cannot describe movements of charged atoms or molecules with one set of rate constants over a range of conditions**.
A proof goes like this:
Consider the spatial series of reactions
X <==>**Y** <==>**Z**
Currents in a series of reactions analyzed by mass action are not (in general) equal:
$I_{XY}/F = z_X k_{XY}$ **[X]**-$z_Y k_{YX}$ **[Y]**; $I_{YZ}/F = z_Y k_{YZ}$**[Y]**-$z_Z k_{ZY}$**[Z]**
Kirchoff's current law requires $I_{XY} = I_{YZ}$ under all circumstances and conditions.
Details can be found at http://arxiv.org/abs/1409.0243 on the physics archive. The artifactual difference I_{XY} - I_{YZ} can have large effects**.** It can produce net charge and electric fields strong enough to break down membranes, proteins, chemical bonds, and even ionize atoms, because of the enormous strength of the electric field, as described unforgettably in p.1-1, of "***Feynman's Lectures on Physics, Vol. 2, Mainly Electromagnetism***…" http://www.feynmanlectures.caltech.edu/II_toc.html.

Voltage-gated Ca Channels

2921-Pos Board B351

Targeting T-Type Channels with Protxii-Like Toxins

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Few gating-modifier toxins have been reported to specifically target T-type calcium channels, and the structural basis of toxin sensitivity remains incompletely understood. Unlike the homotetrameric Kv channels, voltage-gated calcium channels are comprised of four different domains, presenting the possibility of multiple toxin binding sites. Studies of Kv channels identified a S3b-S4 helix-turn-helix motif, termed paddle motif, which moves at